ERGOT PRODUCTION AND IMPROVEMENT

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Ergot of rye of commerce is the sclerotium developed by the fungus *Claviceps purpurea* Tul., on the ears of rye replacing some of the grains. It is an important drug particularly useful in the treatment of *post partum* hæmorrhage. The world's supply of this drug was for a long time obtained from Spain, Portugal or Russia. Natural infection of the rye crop cultivated in these countries was mainly responsible for the availability of the drug in Europe. Rye is not cultivated to any extent in India. The requirements of this drug in India depended entirely on imports. During the World War II there was cessation of the supplies of ergot and it was not available even in several hospitals. At this juncture the Madras Agricultural Department initiated the artificial production of ergot on the Nilgiris (Thomas and Ramakrishnan, 1942) and made it available to Indian pharmaceutical firms. The factors influencing infection and quality of ergot on the Nilgiris were investigated and the results are recorded in this communication.

MATERIALS AND METHODS

A limited area at the Agricultural Research Station, Nanjanad, was being cultivated with a variety of rye whose origin had been traced to Italy. This was the nucleus host material which was multiplied in later years. Other varieties were also obtained from the Agricultural Departments of New South Wales and Kashmir and from the Head of the Division of Botany, Agricultural Research Institute, New Delhi, for comparative trials. Fresh cultures of *Claviceps purpurea* were obtained through the courtesy of Dr. Magee and Mr. I. A. Watson of Australia. These were multiplied for later use. The analyses of sclerotia for determination of the alkaloid content were made according to the method described by Mukerji and De (1944). Inoculation of the rye crop was effected by spraying the spore-suspension on opening flowers with the help of stirrup pumps or Hyject sprayers. Five bottles of the culture were used to spray an acre once. The sclerotia were harvested by hand picking.

EXPERIMENTS

Multiplication of culture of the fungus.—The stock culture of the fungus was maintained on Kirchoff's agar media (1929). Comparison of the relative growths of the fungus on media with asparagin or peptone as the source of nitrogen showed that the latter substance can be used to replace asparagin without any disadvantage. For purposes of spray inoculation the cultures were grown on sterilised rye grains in ordinary pint bottles. The bottles were filled to one-third the capacity with clean rye grains and an equal quantity of water was added. They were plugged and autoclaved for 40 to 45 minutes to ensure complete sterilisation. This was found to be the best medium. Mixtures of equal quantities of wheat and rye or oats and rye also produced good growth of fungus.

Two methods of inoculation of the media in the bottles were tried. In one series bits of agar cultures 0·5 cm. in diameter were placed on the sterilised grains and the bottle was thoroughly shaken to mix the inoculum with the grains. In the second series a heavy suspension of the spores was made by mixing a similar bit of the culture with sterilised water in test-tubes. Two cubic centimetres of this suspension were poured into a bottle of sterilised grains and shaken well. The second method was found to be better. A satisfactory growth developed within a fortnight while in the former it took over a month to grow through the entire medium. Thus the latter method helped to save time.

Altitude and growth of rye.—Rye is a crop of the temperate zone. In order to test the most suitable elevation in this province for the cultivation of rye it was sown in 5 different places, *viz.*, Coimbatore (1,400 ft.), Knoll Estate (Nilgiris, 4,000 ft.), Coonoor (5,600 ft.), Keti (6,500 ft.), Nanjanad (7,000 ft.). In all places the sowings were made in June to July. The plants dried up before reaching the flowering stage at Coimbatore. At Knoll Estate the growth was poor and seed setting unsatisfactory. In Coonoor and higher elevations the growth was very good and the yield of grain was satisfactory. From these observations it was decided that the most suitable places for rye cultivation for ergot production in the province must be at an elevation of 5,600 ft. or over and later sowings were confined to the upper elevations of the Nilgiris and Kodaikanal.

Variety of rye and infection.—A comparison was made of the different varieties of rye available with us to find out the most satisfactory variety for maximum production of ergot. Twelve varieties were under trial. These were grown in replicated quarter-cent. plots as only small quantities of the seed could be obtained and uniformly sprayed with spore suspension. The ergots produced were harvested dried and weighed. The results are recorded in Table I.

TABLE I

Statement showing the nature of growth and yield of ergot of different varieties of rye

Variety	Nature of growth	Average yield of ergot in grams
Af. 31-2 ..	Slow growing few tillers	7
Af. 32-2 ..	do	12
Af. 32-3 ..	do	15
Af. 32-5 ..	do	13
Af. 33-1 ..	do	16
Af. 34-2 ..	do	18
Af. 36-3 ..	do	16
Af. 12-2 ..	do	6
Af. 306 ..	do	15
Kashmir ..	Bushy prostrate-few tillers slow growing	20
Black winter rye ..	Quick growth-erect, satisfactory tillering	98
Local rye ..	Erect, many tillers, taller than black winter rye	88

The Australian black winter rye and the local rye were found to be the most promising varieties for ergot production. Hence for large-scale production of ergot these two varieties only were used.

Time of sowing and infection.—It has been observed in other countries that humid weather favours ergot infection. With a view to find out the most favourable period for the cultivation of rye for ergot production rye was sown in one cent. plots every month beginning from January to December 1943. The plants were inoculated when flower opening commenced by spraying the spore suspension twice a week. Eight sprayings were given to each plot. The ergot was handpicked when mature and the dry weight determined. Ttable II gives the harvest figures.

TABLE II

Statement showing the date of sowing and yield of ergot

Date of sowing	Date of flower opening	Dry weight of ergot in grams	Remarks
6—1—1943	26—4—1943	85	
6—2—1943	20—5—1943	96	
6—3—1943	23—6—1943	140	
6—4—1943	2—7—1943	278	
6—5—1943	13—8—1943	231	
6—6—1943	31—8—1943	198	
6—7—1943	15-10—1943	143	
6—8—1943	17-11—1943	118	
6—9—1943	16-12—1943	98	
6-10—1943	15—2—1944	10	
6-11—1943	15—3—1944	15	Growth sparse few sclerotia
6-12—1943	7—4—1944	5	„

It was observed that good yields were obtained when rye was sown between April and June. But the maximum yields were from April sowings and generally cereals are sown on the hills in April soon after the receipt of summer showers. The results showed that for commercial production of ergot sowings after August were not advisable. An examination of the rainfall records during the flowering period showed a positive correlation between the number of rainy days in the month of flowering and the yield of ergot. In the months of July to October there were 20 or more rainy days in the month in 1943, and the plots which flowered during this period gave the best yield. Between December and April the maximum number of rainy days were only six per month and during the rest of the period the weather was bright and sunny with the result that infection was very low or negligible. Thus it becomes evident that under normal conditions of rainfall it is advantageous to sow in April. Experience gained in later years have brought the close relationship between the number of rainy days during the flowering period and intensity of infection into more prominence. Spells of misty weather with light drizzle have proved very beneficial for spread of infection. But heavy rain during the maturing stages of ergot have resulted in causing lodging of rye and shedding of ergot.

Frequency of spray inoculations and intensity of infection.—The rye field was divided into one cent. plots. The number of spray inoculations was varied from two to eight in different plots. But in all cases the inoculations were commenced only after anthesis. The percentage of infected tillers were recorded in the different plots and the average calculated. It was observed that higher incidence of infection was evident with increase in the frequency of sprays. There was no difference between the plots receiving six sprays and those sprayed eight times. The average percentages of infection are recorded in Table III.

TABLE III

Frequency of sprays and intensity of infection

Number of spray inoculations	Percentage of infection
2	58
4	65
6	70
8	71

When to spray.—The spray inoculations were conducted at different periods after the emergence of the ear in order to determine the proper time for spraying. The plants were raised in isolated plots and the inoculations were made in the first, second, third, fourth and fifth weeks of the emergence of ears outside the shot blade. The infection was very low in the plots which were sprayed in the first or fifth week of emergence. The highest infection occurred in plots sprayed in the third week. It must be mentioned that during this period flower opening was at its peak with the anthers dangling out from most of the spikelets. The presence of open flowers was found to be the most significant factor. In the first week the flowers had not opened. In the fifth week fertilisation was over and grain formation had started. The intensity of infection was proportional to the number of flowers open at the time of spraying.

Pathogenicity trials.—Barger (1931) has given a long list of graminaceous hosts for *Claviceps purpurea.* Atanasoff (1920) has however stated that this fungus exhibited physiologic specialization and the form on one host might not pass on to all the other hosts. In order to determine the probable host range of this fungus on the Nilgiris artificial inoculations were carried out on the following plants, *viz.*, *Avena sativa* L., *Amphilophis foulkesii* C. Fisch., *Anthoxanthum odoratum* L., *Cymbopogon polyneuros* Stapf., *Chrysopogon zeylanicus* Thw., *Cynodon dactylon* Pers., *Digitaria marginata* Link., *Glyceria fluitans* R. Br., *Hordeum hexastichon* L., *Panicum miliare* Lamk., *Paspalum dilatatum* Poir., *Pennisetum clandestinum* Hochst., *Sporobolus indicus* R. Br., *Tripogon bromoides* Roth. *Ischœmum aristatum* L., *Lolium temulentum* L., *Oplismenus compositus* Beauv. and *Themeda triandra* Forsk. The results were in the negative. But few instances of positive infection were obtained after repeated inoculations on *Triticum vulgare* Vill., *Avenastrum asperum* C. Fisch., and *Vulpia myuros* Gmel. Out of 200 ears of wheat inoculated, infection was observed on two ears. Three out of 25 ears of *Avenastrum asperum* and 8 out of 30 ears of *Vulpia* developed small sclerotia.

Alkaloid content of ergot.—The usefulness of ergot depends on its alkaloid content. The sclerotia produced in the first year were assayed by the Research Officer, Madras Medical College, and the quality of the ergot was pronounced to be good (Thomas, etc., 1942). Assay of one sample by Dr. Mukerji (1943) showed that the alkaloid content calculated as ergotoxine was in the neighbourhood of 0·13 per cent. Meanwhile the B. P. standard of raw ergot had been raised from 0·05 to 0·2 per cent. of alkaloids. This change necessitated the improvement of the quality of the Nilgiri produce, One of the methods of doing this was by selection of sclerotia having high alkaloid content and producing cultures of the fungus from such selected sclerotia. The use of these cultures for inoculation led to the stepping up of the alkaloid content of the new crop of ergot (Bekesey, 1940). Sclerotia were cut into two halves. One half was used for the assay and the other utilised for the isolation of the fungus if found to have high alkaloid content. The repetition of this process year after year has led to considerable improvement of the alkaloid content of Nilgiris ergot. The results are shown hereunder.

TABLE IV

Improvement in the alkaloid content of ergot by selection

	Average alkaloid content of bulk produce per 100 gm. of ergot		Highest alkaloid content of individual sclerotia per 100 gm. of ergot (as ergotoxine)
	Ergotoxine	Ergometrine	
1944	190 mgm.	8 mgm.	400 mgm.
1945	300		580
1946	391	91	920
1947	470		1190

The analyses of the bulk produce of 1944 and 1946 were carried out by Dr. Mukerji. The improvement of quality by continuous selection is thus shown to be practicable. The improved cultures were employed for large-scale production.

The influence of the application of manures to the rye crop on the yield and quality of the ergot produced was investigated. The rye was raised on a field which had been previously cropped with potato. Individual plots were manured with bone meal, farmyard manure, fish guano, groundnut cake and ammonium sulphate respectively at two levels. The sowings were made in April 1944. The yield of sclerotia was recorded. The ergot from each plot was analysed separately and the alkaloid content determined. The results showed that there was no significant difference between the treatments, either in yield or in alkaloid content. The figures for yield and alkaloid content were as given in Table V,

TABLE V

Manuring and yield and quality of ergot

Treatment	Average yield of ergot per plot gm.	Alkaloid content per 100 gm. of ergot mgm.
FYM. (5 tons per acre)	117	220
FYM. (10 do)	108	210
Fish guano (1,200 lb. do)	121	190
do (600 do)	111	200
Bone meal (1.000 lb. do)	108	230
do (2,000 lb. do)	117	200
G. N. cake (1,000 lb. do)	119	180
do (2,000 lb. do)	113	200
Amm. sulphate (1 cwt. do)	120	220
do (2 cwt. do)	117	200
Control	110	200

FYM. = Farmyard manure. G.N. cake = Groundnut cake.

GENERAL

A scheme financed by the Madras Government for the large-scale production of ergot has been in operation on the Nilgiris from 1944. These results have been of considerable importance in the selection of the venue for large-scale production of ergot and in deciding the varieties of rye to be grown. During the five years that the scheme has been in progress it has been realised that the most important factor is the synchronisation of the time of flowering with the prevalence of spells of misty weather or light rains. In years when bright weather was experienced during the flowering period with few rainy days (*e.g.*, in 1948) the production of ergot was very disappointing. The yield of ergot has varied according to the attention bestowed on the various operations by the growers. In Sholada (Nilgiris) one farmer was able to harvest 328 pounds of dried ergot from an area of three acres.

Fresh ergot is usually preferred for processing into ergot preparations as it has been reported that it deteriorates in storage with lapse of time. There was occasion to keep in storage over 1,000 pounds of ergot for 18 months. The dry ergot had been packed in paper-lined plywood chests, nailed down and covered over with jute gunnies. The lot had been analysed before storage and the average alkaloid content was 0·39 per cent. After a period of 18 months the stock was opened. The sclerotia were quite normal and no evidence of insect damage or mouldiness was noticed. Samples were taken out and again analysed. The alkaloid content was found to be varying between 0·35 and 0·37 per cent. This was evidence enough to show that when well preserved the deterioration of ergot is not significant.

SUMMARY

Large-scale production of ergot has been in progress, on the Nilgiris, under the auspices of the Madras Government. Rye grows well only above 5,600 ft. The crop sown in April gives highest yield of ergot. Six to eight sprayings are necessary for maximum infection. The larger number of rainy days during the flowering period favours infection. Manures do not have any influence in yield or quality of ergot. Australian black winter rye and local rye are suited for large-scale production of ergot. The pathogenicity trials are described. The quality of the ergot was improved by selection of sclerotia containing a high percentage of alkaloids for obtaining cultures. The alkaloid content of the Nilgiri ergot was raised from 0·19 per cent. to 0·40 per cent.

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